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Comparison of disease severity caused by four soil-borne pathogens in winter cereal seedlings

Ahmed Saad, Bethany Macdonald, Anke Martin, Noel L. Knight, and Cassandra Percy

Abstract. In Australia, crown rot of cereals is predominantly caused by *Fusarium pseudograminearum* and *Fusarium culmorum*, and common root rot by *Bipolaris sorokiniana*. *Fusarium pseudograminearum* is an important pathogen causing Fusarium head blight worldwide and has also been reported to cause crown rot of wheat. The comparative ability of *F. pseudograminearum*, *F. culmorum*, *F. graminearum* and *B. sorokiniana* to cause crown rot and common root rot across a range of winter cereal species requires investigation. In glasshouse trials, we inoculated one cultivar each of barley, bread wheat, durum wheat, oat and triticale with two strains of each of the four pathogens. At 21 days after inoculation, the sub-crown internode and leaf sheaths of each plant were visually rated for brown discoloration. Shoot length and dry weight of inoculated plants were compared with those of non-inoculated controls. Barley and bread wheat had the highest disease severity ratings on leaf sheaths and sub-crown internode (64.7–99.6%), whereas oat had the lowest disease severity ratings across all pathogens (<5%). The shoot length of all cultivars was significantly reduced (by 12.2–55%, *P* < 0.05) when exposed to *F. pseudograminearum*. This study provides a comparison of pathogenicity of crown rot and common root rot pathogens and demonstrates significant variation in visual discoloration and host response across a range of winter cereals.

Keywords: barley, *Bipolaris*, crown rot, *Fusarium*, oat, triticale, wheat.

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Introduction

Crown rot and common root rot of cereals are of key economic significance worldwide (Kumar et al. 2002; Kazan and Gardiner 2018). In Australia, crown rot results in estimated annual losses of AUD$1 million and $79 million for the barley and bread wheat industries, respectively, and common root rot results in estimated annual losses of $13 million and $30 million to these industries (Murray and Brennan 2009, 2010).

*Fusarium pseudograminearum* is the predominant fungus associated with crown rot of cereals in Australia (Burgess et al. 1975; Backhouse et al. 2004), whereas *Bipolaris sorokiniana* causes seedling blight and the disease common root rot (Wildermuth 1986). *Fusarium culmorum* also occurs across the Australian grain belt and has been described as the dominant crown rot pathogen in cooler, high-rainfall areas of South Australia and Victoria (Backhouse and Burgess 2002; Backhouse et al. 2004). *Fusarium graminearum* has been associated with crown rot in the USA (Dyer et al. 2009), South America (Moya-Elizondo et al. 2015) and China (Zhang et al. 2015). In Australia, *F. graminearum* has been reported to cause epidemics of Fusarium head blight (Burgess et al. 1975; Obanor et al. 2013), and although *F. graminearum* strains may cause crown rot of wheat under artificial conditions, they were less aggressive than *F. pseudograminearum* strains (Akinsanmi et al. 2004). *Fusarium graminearum* has been divided into 16 phylogenetically distinct species (Aoki et al. 2012), with *F. graminearum* sensu stricto considered the most important Fusarium head blight pathogen worldwide (Gale et al. 2007; Backhouse 2014). Other *Fusarium* species associated with winter cereals include *F. avenaceum*, *F. crookwellense* and *F. poae*; however, these fungi are infrequently isolated from crown rot diseased tissue (Backhouse et al. 2004; Obanor and Chakraborty 2014). *Bipolaris sorokiniana* and *Fusarium* species such as *F. pseudograminearum*, *F. culmorum* and *F. graminearum* have been reported to occur together in the same paddocks (Smiley et al. 2005; Moya-Elizondo et al. 2011).

Crown rot and common root rot pathogens have been isolated from all small grain and winter cereals including barley (*Hordeum vulgare*), bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. *durum*), oat (*Avena sativa*) and triticale (*× Triticosecale*) (Burgess et al. 2001; Backhouse and Burgess 2002; Kumar et al. 2002). In Australia, extensive research on crown rot has focused on
F. pseudograminearum. Commercial bread wheat and barley cultivars range from moderately susceptible to very susceptible to F. pseudograminearum (Sturgess 2014; Lush et al. 2018). Commercial durum wheat cultivars are considered susceptible to very susceptible to F. pseudograminearum (Lush et al. 2018). Limited research on varietal rankings has been conducted on other winter cereals. Three triticale genotypes (Ningadhu, Hawkeye and Berkshire) have been reported as susceptible to F. pseudograminearum (Klein et al. 1989; Knight and Sutherland 2017), and low or zero levels of disease have been measured in oat cultivars following inoculation with F. pseudograminearum (Nelson and Burgess 1994; Burgess et al. 2001; Percy et al. 2012). Further to varietal rankings, research into F. pseudograminearum has included the infection process of the fungus (Burgess et al. 1975; Wildermuth and McNamara 1994; Percy et al. 2012; Knight and Sutherland 2016), genetic resistance including testing genetic structures, aggressiveness and toxigenicity of F. pseudograminearum populations (Bentley et al. 2008; Obanor and Chakraborty 2014; Khudhair et al. 2019), and yield loss in barley and bread wheat cultivars (Klein et al. 1989; Liu et al. 2012; Kirkegaard et al. 2004).

Barley and wheat cultivars have a wide range of responses to infection with the common root rot pathogen B. sorokiniana (Wildermuth et al. 1992). In Australia, commercial bread wheat cultivars range from moderately resistant to very susceptible (Lush et al. 2018), and significant yield losses have been reported (Wildermuth et al. 1992). The yield loss in susceptible cultivars ranged from 13.9% to 23.9%, whereas yield losses in partially resistant cultivars ranged from 6.8% to 13.6% (Wildermuth et al. 1992). Wildermuth and McNamara (1991) reported an increase in B. sorokiniana populations under wheat, barley and triticale, indicating the potential for these crops to maintain or increase inoculum levels.

The symptoms of crown rot and common root rot disease are similar, and therefore difficult to distinguish without conducting pathogen isolation and identification tests. Crown rot symptoms caused by different Fusarium species are also indistinguishable. The symptoms of crown rot start as small necrotic lesions on the coleoptile, followed by a browning of the sub-crown internode and leaf sheath tissue (Burgess et al. 2001). The first obvious symptom of crown rot in the field is browning of stem bases, which is usually observed after flowering (Burgess et al. 2001). Subsequent discoloration can reach up to the fifth node in stem tissue (Butler 1961; Burgess et al. 2001). Similar to crown rot, common root rot symptoms first appear as small brown necrotic lesions on the coleoptile and roots (Wegulo and Klein 2010). As the disease progresses, lesions also develop on the sub-crown internode and lower parts of the leaf sheaths and the stem (Burrag and Tinline 1960). The sub-crown internode has typically been used for rating common root rot disease, whereas leaf sheaths and stems have been used for rating crown rot (Wildermuth et al. 1992; Burgess et al. 2001). A strong association has been observed between sub-crown internode browning and resistance to common root rot in barley and wheat inoculated with B. sorokiniana (Wildermuth et al. 1992). This has not been demonstrated for F. pseudograminearum crown rot of wheat (Wildermuth and McNamara 1994; Percy et al. 2012).

Although multiple fungal species can be associated with crown rot and common root rot, and barley, bread wheat, durum wheat, oat and triticale have been reported as potential hosts of these fungal species, the comparative ability of these fungi to cause significant crown and common root rot disease across these winter cereal species has not been examined in detail. Knight and Sutherland (2017) and Hollaway et al. (2013) compared the visual discoloration caused by a single strain of each of F. pseudograminearum and F. culmorum across a range of winter cereal species. In these studies, F. culmorum caused discoloration on winter cereals the same as or less than F. pseudograminearum. Furthermore, F. graminearum has not been considered an important pathogen causing crown rot in Australia (Akinsannmi et al. 2004). However, with a changing climate, F. graminearum could be a significant pathogen causing crown rot on hosts such as barley, bread wheat, durum wheat, oat and triticale. Thus, the present study aimed to examine the visual discoloration caused by four crown rot and common root rot pathogens (F. pseudograminearum, F. culmorum, F. graminearum and B. sorokiniana) on leaf sheaths and sub-crown internodes of single, commercially important cultivars of barley, bread wheat, durum wheat, oat and triticale. This study further examined additional host responses to inoculation with the four pathogens through shoot length and dry weight measurements of each cereal species. Knowledge of the disease-causing abilities of each pathogen species informs strategies for disease management in crop rotations and future breeding goals.

Materials and methods

Strains and inoculum preparation

Two strains of each pathogen (F. pseudograminearum, F. culmorum, F. graminearum and B. sorokiniana) were used for inoculations (Table 1). Colonised grain inoculum was produced using a modified method described by Malligan (2009) and Percy et al. (2012). A single spore from each strain was grown on Czapek–Dox agar (CZA) (Leslie and Summerell 2008) and incubated for 7 days at 25°C for the Fusarium species and 22°C for B. sorokiniana. Mycelium was scraped off two CZA plates for each strain and mixed into individual bags (1 kg) of a mix of sterilised (autoclaved twice) grains of bread wheat (650 g) and barley (350 g) and incubated at 25°C in the dark. After 7 days, the bags were shaken manually every 2 or 3 days over 21 days to encourage uniform colonisation of the grain. After the 28-day period, colonised grain was air-dried between sheets of blotting paper and subsequently kept in the dark at 25°C for a further 14 days and stirred every 2 days. Individual inocula were then ground for ~10 s in an electric hammer mill (Christy and Norris 8” Lab Mill; Christy Turner, Ipswich, UK) to pass through a 2-mm sieve. All inoculum bags were sealed and stored at 4°C for future use.

Plant growth and inoculation

Two replicated seedling tests were conducted in a glasshouse at the Department of Agriculture and...
Fisheries, Toowoomba, Queensland. The plant growth medium consisted of self-mulching black Vertosol of the Irving clay soil association, obtained from the Darling Downs, Queensland (Thompson and Beckmann 1959), mixed with river sand (50% sand:50% soil). This mixture was steam-sterilised at 80°C for 40 min and air-dried for 7 days. No fertiliser was added to the mix. The two seedling tests were planted on 5 April and 5 May 2016 (Australian autumn). Three replicate pots each of barley (cv. Grimmett, moderately susceptible to *F. pseudograminearum* (Sturgess 2014; Lush et al. 2018), bread wheat (cv. Livingston, susceptible to *B. sorokiniana* and *F. pseudograminearum*) (Lush et al. 2018), durum wheat (cv. Hyperno, moderately resistant to moderately susceptible to *B. sorokiniana* and susceptible to very susceptible to *F. pseudograminearum*) (Lush et al. 2018), oat (cv. Genie) and triticale (cv. Endeavour) were inoculated individually with two strains of each of the four pathogens. A non-inoculated control treatment was also included for each cereal species. The two experiments were arranged as a randomised complete block design, where each treatment (combination of pathogen, strain and cultivar) was randomly allocated to a pot within each replicate block. The seedling inoculation method described by Wildermuth and McNamara (1994) was used with slight modifications. Briefly, moist soil (280 g, 38% moisture content) was added to pots (5 cm by 5 cm by 10 cm). Fifteen seeds were sown at a depth of 5.5 cm from the top of the pot and covered with a layer of sieved dry soil (160 g). Inoculum (0.45 g) was applied in an even layer on the soil surface of all pots except the non-inoculated control. The inoculum was covered with dry soil (40 g). All pots were placed in a water bath in a glasshouse at 25°C with natural daylengths. The inoculum was activated after 7 days by watering each pot to field capacity (38% moisture content by weight), after which the pots were watered daily up to field capacity. Plants were harvested 21 days after planting, and 10 plants from each pot were rated for disease severity and assessed for shoot length and shoot dry weight.

**Visual discoloration ratings**

The severity of lesions on the sub-crown internode and the first three leaf sheaths was assessed using a 0–100% rating scale based on the visual brown to black discoloration. Rating of each tissue occurred in 5% increments where 0 is no discoloration and 100% is completely discoloured tissue. Following visual discoloration ratings, all roots and the sub-crown internode were removed, and shoot length of each plant was measured from the base of the crown to the tip of the longest leaf. Individual shoots were placed in paper bags and dried in a 65°C oven (UF160; Memmert, Schwabach, Germany) for 48 h, after which dry weights were recorded.

**Data analyses**

The percentage values of visual discoloration on the first three leaf sheaths were added together and divided by three to give a combined leaf sheath percentage. In order to ensure homogeneity of variance, an arcsine square root transformation was applied to the sub-crown internode and the combined leaf sheath rating data. Analysis of each variable was performed using a linear mixed model. The model included fixed effects for pathogen, strain within pathogen, cultivar, experiment, and their interactions. Terms to account for the replicate blocks, pots, and plants within plots were included as random effects, with these variances estimated separately for each experiment. Estimates of variance parameters were generated using residual maximum likelihood (REML) estimation (Patterson and Thompson 1971). Predictions for each trait were generated from their respective models as empirical best linear unbiased estimators (eBLUEs). Where a transformation had been used, predicted means were back-transformed to the original scale, and approximate standard errors were calculated by using the Taylor series approximation. All analyses were performed with ASReml-R (Butler et al. 2009) in the R software environment (The R Foundation, Vienna). Significance of fixed effects was assessed using a Wald test with a significance level of 0.05.

Data for visual discoloration of the leaf sheaths and the sub-crown internode have each been presented graphically in two ways to allow comparison of significant differences detected among cultivars and strains.

**Results**

**Comparison of leaf sheath visual discoloration**

The appearance of visual symptoms caused by the four pathogens on the first three leaf sheaths of each symptomatic cultivar was similar (Fig. 1). A significant

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**Table 1. Location and source of each strain of *Fusarium pseudograminearum*, *F. culmorum*, *F. graminearum*, and *Bipolaris sorokiniana***

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>BRIP accession</th>
<th>Collection year</th>
<th>Collection location</th>
<th>Source of strain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. pseudograminearum</em></td>
<td>Fp1</td>
<td>64949</td>
<td>2009</td>
<td>Emerald, Qld</td>
<td>Crown rot affected stem</td>
</tr>
<tr>
<td></td>
<td>Fp2</td>
<td>64952</td>
<td>2012</td>
<td>Irvingdale, Qld</td>
<td>Crown rot affected stem</td>
</tr>
<tr>
<td><em>F. culmorum</em></td>
<td>Fc1</td>
<td>64973</td>
<td>2010</td>
<td>Unknown, NSW</td>
<td>Crown rot affected stem</td>
</tr>
<tr>
<td></td>
<td>Fc2</td>
<td>64974</td>
<td>2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. graminearum</em></td>
<td>Fg1</td>
<td>64975</td>
<td>2010</td>
<td>Tolga, Qld</td>
<td>Fusarium head blight affected grain</td>
</tr>
<tr>
<td></td>
<td>Fg2</td>
<td>64976</td>
<td>2010</td>
<td>Clifton, Qld</td>
<td></td>
</tr>
<tr>
<td><em>B. sorokiniana</em></td>
<td>Bs1</td>
<td>64970</td>
<td>2005</td>
<td>Moonie, Qld</td>
<td>Common root rot affected sub-crown internode</td>
</tr>
<tr>
<td></td>
<td>Bs2</td>
<td>64972</td>
<td>2006</td>
<td>Wallumbilla, Qld</td>
<td></td>
</tr>
</tbody>
</table>
interaction of experiment, pathogen, strain and cultivar
\((P = 0.017)\) was observed in the leaf sheath ratings
(Supplementary Materials, Table S1, available at the
journal’s website); therefore, the results of the two
experiments could not be combined for analysis and are
presented individually. *Fusarium pseudograminearum*
causually greater visual discoloration than *F. culmorum*,
*F. graminearum* and *B. sorokiniana* in barley
(cv. Grimmett), triticale (cv. Endeavour) and durum wheat
(cv. Hyperno) in both experiments (Fig. 2). The greatest visual
discoloration rating was observed for *F. pseudograminearum*
in Grimmett (99.6%) in both experiments. Some significant
differences were observed between strains of the same
pathogen; for example, strains *Fc2* and *Fg2* had
significantly higher leaf sheath ratings than strain *Fc1* and
*Fg1*, respectively, for Grimmett, Livingston and Endeavour,
and strain *Fp1* had a greater leaf sheath rating than *Fp2*
for Livingston (Fig. 2) in both experiments.

For most of the *Fusarium* spp. strains, the visual
discoloration on Grimmett leaf sheath tissue was
significantly greater than on all other cereals (Fig. S1).
Hyperno (0.09–30%) and Endeavour (0.002–30%) had less
visual discoloration than Grimmett (0.33 to 99.6%) and
Livingston (1.14–51.53%) when infected by all pathogens,
whereas oat cv. Genie exhibited significantly \((P < 0.05)\) less
visual discoloration across all pathogens (0.03–2.5%) (Fig. S1).
The *B. sorokiniana* strains caused low levels of
visual discoloration (<20%) across the cultivars, with
Livingston exhibiting significantly greater visual
discoloration than the other cultivars.

**Comparison of sub-crown internode visual discoloration**

A significant \((P < 0.001)\) pathogen \(\times\) strain \(\times\) cultivar
interaction for the sub-crown internode rating was observed
(Table S2). The greatest visual discoloration ratings were
observed in Grimmett inoculated with *F. pseudograminearum*
strains *Fp1* and *Fp2* (100%), *F. culmorum* strain *Fc2* (97.6%),
and *F. graminearum* strain *Fg2* (59.8%) (Fig. 3). Sub-crown

internode visual discoloration in Livingston inoculated with
*F. pseudograminearum* strains ranged from 32.3% to 64.7%,
with *B. sorokiniana* strains from 16.5% to 58.2%, and a 31%
visual discoloration rating was observed with the *F. culmorum*
strains (Fig. 3). Significant variation between strains was
observed in sub-crown internode visual discoloration of
Grimmett inoculated with *F. culmorum* and *F. graminearum*,
Livingston inoculated with *F. pseudograminearum* and
*B. sorokiniana*, and Genie inoculated with *F. culmorum* (Fig. 3).

Differences among cultivars varied depending on the
pathogen and the strain. Grimmett (0.5–100%) and Livingston
(0.9–64.7%) exhibited the greatest visual discoloration on the
sub-crown internode, whereas Genie (0–12%) and Endeavour
and Hyperno (0–13.7%) had low levels of sub-crown internode
visual discoloration (Fig. S2).

**Shoot length**

The shoot length of cultivars varied significantly \((P < 0.001;\)
Table S3) in response to pathogen inoculation (Fig. 4). In most
instances, the inoculated treatments had a reduced shoot length
compared with the controls. The greatest reduction in
shoot length occurred in all cultivars inoculated with
*F. pseudograminearum*, where shoot length was reduced by
12% for Genie, 13% for Hyperno, 20% for Endeavour, 34.3%
for Livingston and 55% for Grimmett compared with the
control (Fig. 4). Oat cultivar Genie had the lowest levels of
reduction in shoot length across all pathogens (6–12%)
(Fig. 4).

**Shoot dry weight**

A significant interaction of cultivar and strains with
the pathogens was observed for shoot dry weight \((P = 0.035;\)
Table S4). The reduction in dry shoot weight between control
and inoculated plants was significant for Grimmett inoculated
with both *F. pseudograminearum* strains (45.1–57%), *Fc2*
(11.6%) and *Fg2* (10.7%) (Fig. 5). Genie seedlings
inoculated with strains *Fg1* and *Fp2* also had a significant
decreases of 11% and 9%, respectively, in dry shoot weight
compared with the control. The dry shoot weight of Livingston
was significantly lower than the control when inoculated with
*Fp1* (17.2%).

**Discussion**

Our study identified significant variation among visual
discoloration ratings caused by crown rot and common root
rot pathogens on leaf sheath and sub-crown internode tissues
of five winter cereals. To the best of our knowledge, this is the
first direct comparison of disease caused by these four
pathogens across major winter cereal species. This
information will be important for predicting the disease risk
across these cereal species, and the potential implications of
crop rotations in the presence of these pathogens.

*Fusarium pseudograminearum* strains caused greater visual
discoloration than any of the other pathogens on Grimmett,
Livingston, Hyperno and Endeavour. Similar results were
observed by Knight and Sutherland (2017), who reported a
comparison of visual disease symptoms on the leaf sheaths
and fungal biomass of a single strain of each of
*F. pseudograminearum* and *F. culmorum* in seedlings of six winter cereals (barley, durum wheat, oat, rye (*Secale cereale*), spring wheat and triticale) and three summer cereals (maize (*Zea mays*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*)). *Fusarium pseudograminearum* caused greater discoloration than *F. culmorum* in all of the cereals except oat, rye, maize and rice, for which only minimal disease was reported.

In our study, *F. graminearum* caused brown discoloration on the sub-crown internode and leaf sheaths of Grimmett and Livingston at levels similar to *F. culmorum* but less than *F. pseudograminearum. *Fusarium graminearum* has not historically been considered an important crown rot pathogen in Australia (Obanor and Chakraborty 2014). However, Dyer et al. (2009) and Obanor and Chakraborty (2014) suggested that the ability of *F. graminearum* to cause crown rot might increase in areas where Fusarium head blight is more common. In Australia, the fungus has been isolated from the crowns in areas where head blight occurred in northern New South Wales and in warm to subtropical areas with moderate to high rainfall in Queensland (Backhouse and Burgess 2002; Akinsanmi et al. 2004). The

Fig. 2. Mean combined leaf sheath visual discoloration ratings for each experiment × strain × cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains Fp1 and Fp2), *F. culmorum* (strains Fc1 and Fc2), *F. graminearum* (strains Fg1 and Fg2), *Bipolaris sorokiniana* (strains Bs1 and Bs2), and non-inoculated control for each host. Means with the same letter are not significantly different within a cultivar and experiment at α = 0.05.
results support the potential for *F. graminearum* to contribute to crown rot disease in Australia under favourable conditions. However, only *F. graminearum* strains isolated from Fusarium head blight were available in the present study, and along with the difference in visual discoloration caused by each isolate, it suggests that further assessment with strains from crown rot infections should be investigated.

Low visual discoloration ratings were observed on the leaf sheaths of most hosts inoculated with *B. sorokiniana* strains, except for Livingston, which also had the greatest visual discoloration on the sub-crown internode. This finding confirms previous studies suggesting that *B. sorokiniana* is more effective at causing disease on the lower part of the plant (Burrage and Tinline 1960; Wildermuth et al. 1992).

Variation between strains within a pathogen species was observed for combined leaf sheath and sub-crown internode ratings in some of the cultivars. Each of the *F. culmorum* and *F. graminearum* strains caused different levels of visual discoloration across the cultivars. *Fusarium pseudograminearum* has been described to vary in aggressiveness between strains, depending on several factors including farming system, geographical factors, and

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**Fig. 3.** Mean values of the sub-crown internode visual discoloration rating for the pathogen × strain × cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains Fp1 and Fp2), *F. culmorum* (strains Fc1 and Fc2), *F. graminearum* (strains Fg1 and Fg2), *Bipolaris sorokiniana* (strains Bs1 and Bs2), and non-inoculated control for each host. Means with the same letter are not significantly different among pathogens within a cultivar at $\alpha = 0.05$. 

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the genetic diversity of each strain (Akinsanmi et al. 2004). A similar scenario is suggested to occur in the other Fusarium species assessed.

Low levels of visual discoloration were observed in durum wheat cv. Hyperno on the leaf sheaths and the sub-crown internode after inoculation with each of the pathogens. These results contrast with the findings of Knight and Sutherland (2017), where durum wheat cvv. EGA Bellaroi and Jandaroi had visual discoloration ratings similar to the most diseased cultivars of barley. The low level of disease visual discoloration on durum wheat in our study could be due to some cultivars exhibiting resistance in the early stages of growth, with the disease symptoms becoming more pronounced in the advanced stages. Yang et al. (2010) suggested that different genes can be responsible for crown rot resistance at early developmental stages of wheat and barley cultivars, and this resistance might disappear throughout growing stages. Liu et al. (2012) estimated the fungal biomass in barley, bread wheat and durum wheat cultivars at various times (3, 7, 14, 21, 28 and 35 days) post inoculation with F. pseudograminearum. The fungal quantities measured at the six time points of infection were characterised by three distinct phases in each of the cereals. During all stages, the durum wheat cultivar (Wollaroi, highly susceptible) exhibited late and slow fungal accumulation compared with barley cultivars, indicating in the early stages of infection, less disease may also develop. Lush et al. (2018), in ‘2018 wheat varieties, Queensland’ reported that durum wheat cultivars, including Hyperno, are considered susceptible to very susceptible to F. pseudograminearum. Data in this guide are from disease assessed at maturity (Lush et al. 2018). Although differences in the age of plants were minimal between the present study and that of Knight and Sutherland (2017), the growth conditions and cultivars did vary. The inoculation techniques could also have had an impact on disease progression. Knight and Sutherland (2017) applied 6 million conidia/mL to the coleoptile at 14 days after planting, whereas we applied the inoculum as a layer above the seeds, which required activation by moisture 7 days after planting. Thus, in the present study, the coleoptile grew through the soil and the inoculum, similar to a natural field infection, compared with direct application of a conidial suspension.

Triticale cv. Endeavour had low levels of visual symptoms on the leaf sheaths and sub-crown internode compared with Grimmett and Livingston. Knight and Sutherland (2017) reported that triticale cvv. Hawkeye and Berkshire had a high level of visual discoloration, with responses to a single strain of each of F. pseudograminearum and F. culmorum similar to barley, durum wheat and spring wheat cultivars. This difference may be due to the different inoculation methods used or genetic variation among cultivars.

Oat is considered a resistant or an asymptomatic host of F. pseudograminearum (Percy et al. 2012; Knight and Sutherland 2017). Low levels of visual discoloration, still

Fig. 4. Mean values of shoot length for the pathogen x cultivar interaction. Treatments include Fusarium pseudograminearum (Fp), F. culmorum (Fc), F. graminearum (Fg), Bipolaris sorokiniana (Bs), and non-inoculated control. Means with the same letter are not significantly different within a cultivar at $\alpha = 0.05$. 

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![Graph showing shoot length for different pathogen/cultivar combinations](image-url)
significantly greater than the controls, were observed on the sub-crown internode of Genie when inoculated with one strain of *F. pseudograminearum* and on the leaf sheaths when inoculated with one strain of *F. pseudograminearum*. Although disease levels were low, the capacity of oats to exhibit discoloration after inoculation with *F. pseudograminearum* and *F. culmorum*

**Fig. 5.** Mean values of cultivar dry shoot weight for the pathogen × strain × cultivar interaction. Treatments include *Fusarium pseudograminearum* (strains Fp1 and Fp2), *F. culmorum* (strains Fc1 and Fc2), *F. graminearum* (strains Fg1 and Fg2), *Bipolaris sorokiniana* (strains Bs1 and Bs2), and non-inoculated control for each host. Means with the same letter are not significantly different within a cultivar α = 0.05.
supports recommendations that oat should not be used as a rotational crop for crown rot caused by *F. pseudograminearum* (Nelson and Burgess 1994) or for common root rot management (Wildermuth and McNamara 1991).

The physiological impact of disease caused by these four crown rot and common root rot pathogens has not been extensively detailed. *Fusarium pseudograminearum* resulted in the greatest reduction of shoot length across all cultivars. Grimmett and Livingston had significant shoot length reductions across all pathogens, except in the case of *B. sorokiniana* with Grimmett. Oat cv. Genie had low or no symptoms; however, there was a significant reduction in the shoot length of Genie across all pathogens (P < 0.05). The reduction in shoot length indicates that each pathogen had a negative effect on the development of the host. Similarly, Smiley et al. (2005) indicated that visual discoloration was negatively correlated with plant height for *F. pseudograminearum*, *F. culmorum* and *F. graminearum* but not for *B. sorokiniana*.

Differences in host shoot dry weight were observed but varied according to pathogen or strain. Strain *fpI* significantly reduced dry shoot weight in Grimmett and Livingston, whereas *Pgi* significantly decreased dry shoot weight in Genie (P < 0.05). This outcome contrasts with the findings of Knight et al. (2012), who indicated a significant increase in dry weight of the individual leaf sheath up to the fourth leaf for four bread wheat cultivars colonised by *F. pseudograminearum*, compared with non-inoculated controls. This difference between the two studies could be due to the dry shoot weight of the entire seedling, including leaf blade, being included in our study. A negative impact on the dry shoot weight of plant tissue indicates that each pathogen has a detrimental effect on plant growth; however, this level in reduction was not as significant as that for shoot length. Further assessment of plant height and weight in the field is crucial for investigating the physiological impact of these pathogens.

This study identified significant differences in visual discoloration caused by infection with *F. pseudograminearum*, *F. culmorum*, *F. graminearum* and *B. sorokiniana* in five winter cereals species. *Fusarium pseudograminearum* caused the greatest visual discoloration on both the sub-crown internode and leaf sheaths, followed by *F. culmorum*, *B. sorokiniana* and *F. graminearum*. The most severe disease symptoms were observed on Grimmett and Livingston, whereas Genie showed low or no symptoms. Significant differences were observed in the host response (shoot dry weight and shoot length) to all pathogens, with the reduction in shoot length being more significant than the reduction in shoot weight. The reactions observed across the cereal hosts demonstrate the comparative disease impacts of each of these fungi, which will inform improved management strategies for crown rot and common root rot diseases by crop rotation. A field test will facilitate further investigation of the impact of these four pathogens on the five winter cereals at different stages of plant growth.

**Conflicts of interest**
The authors declare no conflict of interest.

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**References**
Klein T, Burgess L, Ellison F (1989) The incidence of crown rot in wheat, 
Knight NL, Sutherland MW (2017) Assessment of 
Lincoln Extension, 
wheat industry. Australasian Plant Pathology 38, 558–570. doi:10.1071/ 
AP09053 
barley industry. Australasian Plant Pathology 39, 85–96. doi:10.1071/ 
AP09064 
Nelson K, Burgess L (1994) Reaction of Australian cultivars of oats and 
barley to infection by Fusarium graminearum Group 1. Australian 
Journal of Experimental Agriculture 34, 655–658. doi:10.1071/ 
EA9940655 
Obanor F, Chakraborty S (2014) Aetiology and toxigenicity of Fusarium 
graminearum and F. pseudograminearum causing crown rot and head 
blight in Australia under natural and artificial infection. Plant 
Pathology 63, 1218–1229. doi:10.1111/ppa.12200 
Fusarium graminearum and Fusarium pseudograminearum caused the 
Patterson HD, Thompson R (1971) Recovery of inter-block information when 
block sizes are unequal. Biometrika 58, 545–554. doi:10.1093/ 
bioet/58.3.545 
Percy CD, Wildermuth GB, Sutherland MW (2012) Symptom 
development proceeds at different rates in susceptible and partially 
Smiley RW, Gourlie JA, Easley SA, Patterson LM (2005) Pathogenicity of 
fungi associated with the wheat crown rot complex in Oregon and 
Sturgess J (2014) Barley: planting and disease guide 2014 Queensland and 
NSW. AgriScience Queensland, Department of Agriculture Fisheries 
and Forestry, Brisbane, Qld. 
Thompson C, Beckmann GG (1959) Soils and land use in the Toowoomba 
area, Darling Downs, Queensland. Soil and Land Use Series No. 28. 
Division of Soils, Commonwealth Scientific and Industrial Research 
Organization, Melbourne, Vic. 
Wegulo S, Klein R (2010) Common root rot and Fusarium foot rot of 
wheat. NebGuide, University of Nebraska–Lincoln Extension, 
Institute of Agriculture and Natural Resources, Lincoln, NE, USA. 
Wildermuth G (1986) Geographic distribution of common root rot and 
Bipolaris sorokiniana in Queensland wheat soils. Australian Journal of 
Experimental Agriculture 26, 601–606. doi:10.1071/EA860601 
Wildermuth G, McNamara R (1991) Effect of cropping history on soil 
populations of Bipolaris sorokiniana and common root rot of wheat. 
AR9910779 
resistance to crown rot caused by Fusarium graminearum group 1. 
Plant Disease 78, 949–953. doi:10.1094/PD-78-0949 
cased by common root rot in wheat cultivars in Queensland. 
Australian Journal of Agricultural Research 43, 43–58. doi:10.1071/ 
AR9920043 
Yang X, Ma J, Li H, Ma H, Yao J, Liu C (2010) Different genes can be 
responsible for crown rot resistance at different developmental stages of 
wheat and barley. European Journal of Plant Pathology 128, 
495–502. doi:10.1007/s10658-010-9680-3 
Zhang XX, Sun HY, Shen CM, Li W, Yu HS, Chen HG (2015) Survey of 
Fusarium spp. causing wheat crown rot in major winter wheat growing 
PDIS-04-14-0422-RE

Handling Editor: Robert Park

www.publish.csiro.au/journals/cp

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United States. Phytopathology 97, 1434–1439. doi:10.1094/PHYTO- 
97-11-1434 
Yield loss in cereals, caused by Fusarium culmorum and F. 
pseudograminearum, is related to fungal DNA in soil prior to 
planting, rainfall, and cereal type. Plant Disease 97, 977–982. 
doi:10.1094/PDIS-09-12-0867-RE 
Kazan K, Gardiner DM (2018) Fusarium crown rot caused by Fusarium 
pseudograminearum in cereal crops: recent progress and future 
mpp.12639 
Khudhair M, Thatcher L, Gardiner D, Kazan K, Roper M, Aitken E, 
Obanor F (2019) Comparative analysis of genetic structures and 
aggressiveness of Fusarium pseudograminearum populations from 
two surveys undertaken in 2008 and 2015 at two sites in the wheat 
belt of Western Australia. Plant Pathology 68, 1337–1349. 
doi:10.1111/ppa.13056 
Kirkegaard JA, Simpfendorfer S, Holland J, Bambach R, Moore KJ, 
Rebetzke GJ (2004) Effect of previous crops on crown rot and yield 
of durum and bread wheat in northern NSW. Australian Journal of 
Agricultural Research 55, 321–334. doi:10.1071/AR03178 
Klein T, Burgess L, Ellison F (1989) The incidence of crown rot in wheat, 
barley and triticale when sown on two dates. Australian Journal of 
Experimental Agriculture 29, 559–563. doi:10.1071/EA9890559 
Knight NL, Sutherland MW (2016) Histopathological assessment of 
Fusarium pseudograminearum colonization of cereal culms during 
crown rot infections. Plant Disease 100, 252–259. doi:10.1094/ 
PDS-04-15-0476-RE 
Knight NL, Sutherland MW (2017) Assessment of Fusarium pseudograminearum and F. culmorum biomass in seedlings of 
doi:10.1094/PDS-12-16-1739-RE 
Knight NL, Sutherland MW, Martin A, Herde DJ (2012) Assessment of 
infection by Fusarium pseudograminearum in wheat seedling tissues 
using quantitative PCR and a visual discoloration scale. Plant Disease 96, 1661–1669. doi:10.1094/PD-12-11-1050-RE 
cytological and molecular approaches towards better control. 
2002.00120.x 
Vols 2 and 10. (John Wiley & Sons: New York) 
Different tolerance in bread wheat, durum wheat and barley to 
Queensland. Crop Variety Guides. Grains Research and Development 
Corporation, and Queensland Department of Agriculture and Fisheries, 
Brisbane, Qld. 
Malligaid CD (2009) Crown rot (Fusarium pseudograminearum) symptom 
development and pathogen spread in wheat genotypes with varying 
disease resistance. PhD thesis, University of Southern Queensland, 
Qld, Australia. 
dynamics between Fusarium pseudograminearum and Bipolaris 
sorokiniana in wheat stems using real-time qPCR. Plant Disease 95, 
1089–1098. doi:10.1094/PDIS-11-10-0794 
Distribution and prevalence of crown rot pathogens affecting wheat 
crops in southern Chile. Chilean Journal of Agricultural Research 75, 
78–84. doi:10.4067/S0718-58392015000100011

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